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Please find below and/or attached an Office communication concerning this application or proceeding.

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BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

Application Number: 10/816,099
Filing Date: March 31, 2004
Appellant(s): VARADI ET AL.

Ms. Jean Lockyer
For Appellants

EXAMINER'S ANSWER

This is in response to the appeal brief filed on October 18, 2010 appealing from the Office action mailed on December 16, 2009.

(1) Real Party in Interest

The examiner has no comment on the statement, or lack of statement, identifying by name the real party in interest in the brief.

(2) Related Appeals and Interferences

The examiner is not aware of any related appeals, interferences, or judicial proceedings which will directly affect or be directly affected by or have a bearing on the Board's decision in the pending appeal.

(3) Status of Claims

The following is a list of claims that are rejected and pending in the application: 1-8, 10-13, 22 and 23.

(4) Status of Amendments After Final

The examiner has no comment on the appellant's statement of the status of amendments after final rejection contained in the brief.

(5) Summary of Claimed Subject Matter

The examiner has no comment on the summary of claimed subject matter contained in the brief.

(6) Grounds of Rejection to be Reviewed on Appeal

The examiner has no comment on the appellant's statement of the grounds of rejection to be reviewed on appeal. Every ground of rejection set forth in the Office action from which the appeal is taken (as modified by any advisory actions) is being maintained by the examiner except for the grounds of rejection (if any) listed under the subheading "WITHDRAWN REJECTIONS." New grounds of rejection (if any) are provided under the subheading "NEW

GROUNDS OF REJECTION."

(7) Claims Appendix

The examiner has no comment on the copy of the appealed claims contained in the Appendix to the appellant's brief.

(**8) Evidence Relied Upon**

6,124,110	WÖBER et al.	9-2000
5,625,036	HAWKINS et al.	04-1997

Váradi et al., "Monitoring the bioavailability of FEIBA with a thrombin generation assay," J Thrombosis and Heamostasis 1:2374-2380, 2003.

5,952,198	CHAN	09-1999
6,074,826	HOGAN et al.	06-2000
6,576,422	WIENSTEIN et al.	06-2003
6,756,019	DUBROW et al.	06-2004

(9) Grounds of Rejection

The following ground(s) of rejection are applicable to the appealed claims.

Claims 1-8, 10-13, 22 and 23 are rejected under 35 U.S.C. 103(a) as being unpatentable over Wöber et al. (US 6,124,110) in view of Hawkins et al. (US 5,625,036), Váradi et al. ("Monitoring the bioavailability of FEIBA with a thrombin generation assay," J Thrombosis and Heamostasis 1:2374-2380, 2003), Chan (US 5,952,198), Hogan et al. (US 6,074,826), Weinstein et al. (US 6,576,422) and Dubrow et al. (US 6,756,019). This rejection has been discussed in the previous Office actions.

To reiterate, Wöber et al. disclose reagents and an assay for measuring thrombin generation in plasma samples. They disclose natural tissue factor (TF) as a dry powder and solutions of the phospholipids phosphatidylserine (PS) and phosphatidylcholine (PC). Wöber et

al. also disclose that these three reagents are combined to prepare a solution of vesicles or liposomes containing TF, i.e., a TF/PL complex. The ratio of PC to PS in this complex is 6:4. This solution may be frozen in assay portions (see col. 2, lines 15-26; col. 3, lines 9-16, and col. 4, lines 13-46). Wöber et al. also disclose a thrombin standard that is used in their assay (see col. 5, lines 20-40) (see claims 1, 6, 7, 10, 22 and 23). Wöber et al. do not disclose that this complex is lyophilized or the concentrations of TF and PL in the complex.

Hawkins et al., however, disclose that the TF/PL solution may be lyophilized. The ratio of PC to PS in Hawkins et al. is 7:3 (see Example 2 in col. 8 and Example 4 in cols. 9 and 10). The ratio of TF to PL is 1:2000 to 1:20,000, and the PL concentration is 1-300 μM (see col. 4, lines 34-50). At a PL concentration of 1 μM, the TF concentration is 50-500 pM. Synthetic (recombinant) or natural TF may be used, and synthetic or natural phospholipids may be used. Combinations of lipids other than PC and PC may be used (see col. 4, lines 34-62, and col. 5, lines 3-10) (see claims 1-5).

One of ordinary skill in the art at the time of the invention would have been motivated to lyophilize the TF/PL preparation of Wöber et al., because Hawkins et al. teach that this preparation is made as a reagent for performing assays to measure prothrombin time (see Title). This reagent is meant to be used for large-scale clinical assays and is designed to have minimal variability from lot to lot (see col. 1, line 36, to col. 2, line 7. One of ordinary skill in the art would have recognized that a manufacturer of such a reagent would have lyophilized it to reduce the weight and volume for shipping purposes and to impart stability to the reagent. Dry materials are less susceptible to degradation than their liquid form, e.g., as with powdered vs. liquid milk.

One of ordinary skill in the art would have been motivated to prepare a TF/PL complex with the TF and PL concentrations and with the phospholipid ratios disclosed in Hawkins et al.,

because Hawkins et al. teach that these are suitable amounts for preparing a reagent for an assay for measuring prothrombin time. Prothrombin is a thrombin precursor in the clotting pathway (cleaved by the protease prothrombinase to form thrombin). Thus a reagent for measuring prothrombin time may also be used to measure thrombin time.

Regarding the lyophilized thrombin substrate and $CaCl_2$ preparation, Wöber et al. disclose a dry chromogenic thrombin substrate, S 2238 (Chromogenix, now Diapharma) that is soluble in water and that the thrombin reaction is initiated by the addition of $CaCl_2$ to the assay samples (see col. 5, lines 10-25). One of ordinary skill in the art would have been motivated to prepare a lyophilized reagent containing thrombin substrate and $CaCl_2$, because Hawkins et al. teach the advantages of lyophilized reagents in clinical assays. As noted above, the artisan of ordinary skill would have recognized that aqueous solutions can be lyophilized to reduce bulk and improve stability. One of ordinary skill in the art would also have recognized that the thrombin substrate and $CaCl_2$ are both soluble in water or buffer, as disclosed by Wöber et al., and they would have been combined because an enzymatic reaction may also be initiated by the addition of substrate, as well as by the addition of a catalytic substance or cofactor. In an assay of a number of samples, the enzymatic reaction is initiated by the addition of a reagent, ideally simultaneously to all samples, but this reagent may contain the substrate and the cofactor. Combining the substrate and the cofactor reduces the number of pipetting steps, thereby reducing the chance of assay errors due to pipetting errors, and reduces the number of assay steps, allowing the assay to be performed faster. Because the thrombin substrate and $CaCl_2$ are both soluble in water or buffer, one of ordinary skill in the art would have recognized that a solution containing both of these substances would have been prepared and lyophilized. Moreover, Hogan et al. teach that, in a diagnostic kit, or when performing an assay with a diagnostic kit, the reagents may be premixed before lyophilization so that, when reconstituted, a

complete mixture is formed with the reagents in the proper ratio and ready for use (see col. 37, lines 14-29) (see claim 1).

Regarding a thrombin substrate containing a fluorescent label, as noted above, Wöber et al. disclose a substrate containing a colored label. Determinations of thrombin generation are made by measuring the extinction over five minutes at one minute intervals at 405 nm (see col. 5, lines 33-40). Váradi et al., however, disclose a thrombin substrate for a thrombin generation assay that contains a fluorescent label, Z-Gly-Gly-Arg-AMC. An assay reagent comprising 1 mM thrombin substrate and 15 mM calcium chloride was prepared. In each assay sample, 10 µl of a preparation containing a TF/PL complex (the complex containing 17.9 pM TF and 3.2 µM PL, the PL being PC:PS, 80:20) was added to 50 µl of the thrombin substrate solution, and 40 µl of plasma was added to start the reaction. In the assay samples, increases in fluorescence were measured every minute over 2 hours at 460 nm (see p. 2375, Thrombin generation assay). One of ordinary skill in the art would have been motivated to use the thrombin substrate of Váradi et al. as the thrombin substrate in the set of reagents disclosed by Wöber et al., i.e., a fluorescent label instead of a colored label, because Váradi et al. teach that their substrate is available as a dry powder that is soluble in the buffers used in a thrombin generation assay. Thus, lyophilized forms of these powders may also be prepared. One of ordinary skill in the art would have recognized that these substrates are interchangeable with the substrate of Wöber et al., as it would have been well within his capability, when performing a thrombin generation assay, to measure the amount of fluorescence produced over time by a thrombin reaction product instead of the amount of color generated over time by a thrombin reaction product. Both fluorometric and spectrophotometric measurements are standard assay techniques (see claims 1-3, 6, 7, 22 and 23).

With respect to claim 8, which recites phospholipids comprising PC, PS and PE

(polyethanolamine) in a ratio of about 60:20:20 to about 78:17:5, the composition of phospholipid mixtures and the ratios of the different lipid components are result-effective parameters which were routinely optimized by one of ordinary skill in the art. Thus, the claimed variations in Applicants' composition with respect to these parameters clearly would have been obvious at the time of Applicants' invention, the optimization of these parameters being well within the capabilities of the artisan of ordinary skill at the time of Applicants' invention. Additionally, liposomes comprising such a lipid mixture were known at the time of Applicants' invention. Chan (US 5,952,198) discloses liposomes of PC, PS, and PE in a ratio of 4:1:1 that are added to the medium of 293S cells (human embryonic kidney cells) to increase the production of recombinant Factor VIII, a clotting factor. Liposomes of PC, PS and PE in a ratio of 8:1:1 and 16:2:1 are also disclosed (see Table 1, cols. 3 and 4). It is thought that these liposomes stabilize the recombinant Factor VIII in an in vitro medium (see col. 1, lines 49-52). The liposomes with a ratio of 4:1:1 and 16:2:1 are close to those in Applicants' claimed range. One of ordinary skill in the art would have been motivated to use liposomes comprising PC, PS and PE in a ratio of about 60:20:20 to about 78:17:5 instead of liposomes comprising PC and PS in a ratio of 6:4 in a complex with TF because Chan teaches that the PC:PS:PE liposomes have a stabilizing effect on clotting factors, Factor VIII and von Willebrand factor. As noted above, Hawkins discloses that lipid mixtures other than PC:PS, 6:4, may be used in the TF/PL complex.

Regarding a solid support, such as microtiter plate, with lyophilized assay reagents coated onto the wells or assay vessels of the support, and a method of performing an assay using this solid support, Weinstein et al. disclose an assay method using a solid support such as a microtiter plate in which lyophilized detection reagents are immobilized on the solid support (see col. 16, lines 10-25, and col. 17, lines 5-6). The advantages of performing an assay with

this solid support are that the assay is fast and simple and designed for screening a large number of samples (see col. 17, lines 22-30). Dubrow et al. also disclose performing an assay with minute amounts of lyophilized reagents immobilized on a solid support (see col. 12, line 59, to col. 13, line 11). Current trends in biochemical analyses have been toward miniaturization, particularly in microfluidics systems (which manipulate microtiter plates), and which have the advantages of small amounts of reagents needed, faster throughput, automation and improved data (see col. 1, lines 12-19). One of ordinary skill in the art would have been motivated to prepare a kit for measuring thrombin generation comprising assay reagents lyophilized and immobilized on a solid support, because Hawkins et al. teach the advantages of lyophilized reagents, and Weinstein et al. and Dubrow et al. teach the advantages of formulating these reagents as a lyophilized coating on a solid support. Weinstein et al., more specifically, teach the advantages of formulating assay reagents as a lyophilized coating on the wells of a microtiter plate. The cited references also teach the benefits of performing assays with these solid supports (speed, simplicity, high throughput, improved data) (see claims 11-13).

(10) Response to Argument

In part (A), Appellants assert that their invention is not obvious, because of their unexpected result that it is not necessary to add an organic solvent (DMSO) to the lyophilized thrombin substrate reagent, a mixture of the fluorescently labeled peptide Z–G-G-R-AMC and calcium chloride (AMC is 7-amino-4-methylcoumarin and Z is a carboxybenzyl group).

In reply, the instant claims are kit claims and a method of using the kit. The heart of the rejection is that the second reagent in the kit, recited in claim 1, part (ii), is obvious. The feature that this reagent can be dissolved in water to a final concentration of 1 mM peptide and 15 mM calcium chloride is a wherein clause and a method step that is an intended use in a product claim. This wherein clause does not materially change the product, as the product can be

dissolved in any liquid that is compatible with performing a thrombin assay, an assay that measures thrombin generation time. Moreover, Table 1 on p. 14 of the Brief shows that all of the re-dissolved lyophilized samples are dissolved in a liquid that yields a final DMSO concentration of 2%. Thus, it appears that Appellants used a 2% solution of DMSO in water to dissolve their samples, not plain water.

In part (B), Appellants note that, although the disclosure of Váradi et al. is Appellants' invention, Appellants are not arguing whether or not they can disqualify the reference as prior art in this Brief. Appellants note that a rejection under 35 USC § 102 has not been made.

In reply, an anticipation rejection was not made, because the instant claims are kit claims. As discussed above, Váradi et al. teach a thrombin assay that uses the individual reagents in the kit- TF-containing liposomes and a solution of 1 mM AMC-labeled peptide and 15 mM calcium chloride. But, Váradi et al. do not teach how these reagents are made, and they do not teach a kit. A portion of the rejection discusses that lyophilizing reagents and packaging them together in a kit is conventional in the art of biotechnology.

In part (C), Appellants assert that Examiner made an "obvious to try rejection." Appellants assert that the rejection did not explain how each part of the invention is taught by the prior art and that the rejection did not provide motivation for modifying the references to arrive at the invention.

In reply, an "obvious to try rejection" was never made. The way in which each part of the claimed invention is taught by the prior art is explained in full detail in the rejection above, and motivation to combine the references was provided, as discussed above. In short, the first reagent, the TF-containing liposomes, are taught by Wöber et al., while the ratios of the different phospholipids in the liposomes are taught by Chan. The limitations related to mixing reagents together, lyophilizing reagents and packaging them together are taught by Hawkins et al. and

Hogan et al. The teachings of Váradi et al., related to a specific, sensitive and reliable assay using the labeled thrombin substrate mixed with calcium chloride are discussed above. Weinstein et al. and Dubrow et al. teach the advantages of using very small amounts of lyophilized reagents immobilized on solid supports.

In part (C.1.), Appellants discuss the point of novelty of the invention, that one of ordinary skill in the art at the time of the invention would not have expected the reagent of claim 1, part (ii), i.e., the second reagent, the lyophilized mixture of AMC-labeled peptide and calcium chloride to be soluble in aqueous solution or in water.

In reply, Appellants' arguments are confusing, because they use the terms "aqueous solution" and "water" interchangeably. It is not clear if, to dissolve their second reagent, Appellants use water, or a 2% solution of DMSO in water, or a solution of DMSO in water in which the ratio of peptide to DMSO is 1 mM peptide to 2% DMSO. That is, a 5 mM peptide solution (final concentration) requires 10% DMSO in water. It is not clear if lyophilization conditions are such that the DMSO does not evaporate (as noted in the previous Office actions, DMSO has a boiling point of 189 °C), so that plain water can be used to make the solutions, or if DMSO is present in the water used to make the solutions. As noted above, Table 1 on p. 14 indicates that 2% DMSO in water is used to make the solutions. Moreover, as noted above, the claimed composition is considered to be obvious over the prior art. The purported point of novelty is an intended use step in a product claim that does not materially change the product.

In part (C.2.), Appellants assert that there is no reasonable expectation of success in making a clear solution by dissolving their second reagent in an aqueous buffer or water or an aqueous solution. Appellants assert that the reason is because, when the labeled peptide alone, in 10% DMSO in water is lyophilized, and when it is reconstituted by adding a solution that is 15 mM calcium chloride in water, for a final concentration of 1 mM peptide and 2%

DMSO, a cloudy suspension forms that requires heating and vigorous stirring for solution. But, when an aqueous solution of the labeled peptide in 2% DMSO and 15 mM calcium chloride is lyophilized, a clear solution is formed when water is added, for a final concentration of 1 mM peptide and 2% DMSO. The HEPES-containing buffer improves the solubility of the peptide.

In reply, again, Appellants' arguments are confusing, because the terms aqueous buffer, water, and aqueous solution are used synonymously in the context of solvents for the second reagent. It cannot be determined what Appellants did in their experiments. The only unexpected result is that Sample 1 did not form a clear solution. The other results are expected results. If the labeled peptide, at a concentration of 1 mM, is known to be soluble in 2% DMSO, one would expect a clear solution when redissolving it in 2% DMSO to a final concentration of 1mM (Sample 3a). As for Samples 3b and 4, HEPES is a surfactant, and a surfactant would be expected to increase the solubility in water of a hydrophobic or lipophilic molecule. As discussed above, however, to reiterate, the rejection is that the claimed invention, the claimed product, is obvious. The feature that the second reagent can be dissolved in water to a final concentration of 1 mM peptide and 15 mM calcium chloride is a wherein clause and an intended use step in a product claim. The wherein clause does not materially change the product, as the product can be dissolved in any liquid that is compatible with performing a thrombin assay. For example, as previously discussed, in the assay of Váradi et al., the assay samples were mixtures of 10 µl of Appellants' first reagent (TF-containing liposomes), 50 µl of Appellants' second reagent (1 mM labeled peptide and 15 mM calcium chloride) and 40 µl of plasma (see p. 2375, right col.). Even if the second reagent was dissolved in 2% DMSO instead of water, Váradi et al. disclose that their assay is successful, as well as superior to earlier assays, which measure prothrombin generation time or clotting time and cannot be used accurately in people taking Factor VIII inhibitors, such as hemophiliacs (see pp. 2374-2375).

In part (C.3.), Appellants assert that the invention as a whole, not as its parts, should be considered for obviousness. Yet, Appellants assert again that the reason that the invention is not obvious is because the artisan of ordinary skill would not have expected the second reagent to be soluble in water. In reply, this point is addressed several times above.

In part (C.4.), Appellants assert that claims 22-23, drawn to a method of using the claimed product, are separately patentable. Yet, Appellants assert again that the reason that these claims are separately patentable is because the artisan of ordinary skill would not have expected the second reagent to be soluble in an aqueous solution that does not contain an organic solvent. In reply, this point is addressed several times above.

(11) Related Proceeding(s) Appendix

No decision rendered by a court or the Board is identified by the examiner in the Related Appeals and Interferences section of this examiner's answer.

For the above reasons, it is believed that the rejections should be sustained.

Respectfully submitted,

/Rosanne Kosson/, Examiner

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